B1 conclude

(b) repeatedly replicating the cells from step (a) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progency cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

B2 9/03

18. (Amended) A method of integrating multiple copies of exogenous DNA into reiterated chromosomal DNA of cells, comprising:

(i) transforming yeast cells with a replicative and integrative plasmid comprising an autonomous replicating sequence, exogenous DNA, and a selection marker, the exogenous DNA being flanked on each end by a DNA sequence homologous to a reiterated sequence of DNA of the host;

(ii) repeatedly replicative the transformed yeast cells from step (i) to produce a number of generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative plasmid in subsequent generations of the progeny cells and result in progeny cells each containing multiple integrated copies of the exogenous DNA; and

(iii) replicating the progeny cells from step (ii) to produce a number of generations of progeny cells in the absence of selection for cells which include the selection marker, so as to promote the loss of the plasmid in subsequent generations of progeny cells and recover yeast cells each containing multiple copies of the exogenous DNA integrated into its chromosomal DNA.

is this more than one?

?

is it lost?

B3 9/05

25. (Amended) A yeast which ferments xylose to ethanol, comprising:

which is

a yeast having multiple copies of exogenous DNA integrated into chromosomal DNA of the yeast, the exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase, the yeast fermenting xylose to ethanol and substantially

retaining its capacity for fermenting xylose to ethanol when cultured under non-selective conditions for at least 20 generations.

28. (Amended) A plasmid vector comprising a functional yeast autonomous replicating sequence and an exogenous DNA comprising a first selection marker, the exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further including a second section marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a target yeast cell.

29. (Amended) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA including genes encoding xylose reductase, xylitol dehydrogenase, and xylulokinase flanked on each end by a DNA flanking sequence which is homologous to a reiterated DNA sequence of the target yeast cell, the plasmid vector for use in integrating the exogenous DNA sequence into chromosomal DNA of a yeast to form stable integrants which ferment xylose to ethanol.

30. (Amended) A method for forming cells having multiple integrated copies of an exogenous DNA fragment, comprising:

replicating cells having reiterated genomic DNA and which contain a replicative and integrative plasmid comprising an autonomous replicating sequence and containing the exogenous DNA to produce multiple generations of progeny cells while selecting for cells which include the selection marker, so as to promote the retention of the replicative and integrative plasmid in subsequent generations of the progeny cells and produce progeny cells having multiple integrated copies of the exogenous DNA.

31. (New) The yeast of claim 1 wherein the yeast maintains xylose fermenting capability after culture in non-selective medium.

32. (New) The method of claim 14 wherein the cells are yeast.

33. (New) The method of claim 30 wherein the cells are yeast.

34. (New) A plasmid vector comprising a functional yeast autonomous replicating sequence and exogenous DNA flanked on each end by a DNA flanking sequence which is homologous to a reiterated ribosomal DNA sequence of the target yeast cell, the plasmid further comprising a selection marker in a position other than between the DNA flanking sequences, the plasmid vector for use in integrating an exogenous DNA sequence into chromosomal DNA of a target yeast cell.